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Geostatistical analysis of the spatial distribution of *Rotylenchulus reniformis* on cotton cultivated under crop rotation

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Summary. The spatial distribution of *Rotylenchulus reniformis* on cotton cultivated in crop rotation with sorghum-peanut-velvetbean was studied using geostatistics. The experimental field, which had been continuously cropped with cotton for 20 years, comprised two 32 x 48 m-grids, each divided in sixty-four 4 x 6 m sampling plots. For all crops, 300 cm^3 soil samples were taken at the center of each plot at crop germination (Pi) and again at harvest (Pf), from which the numbers of nematodes were determined. The results revealed that the spatial distribution of *R. reniformis* was highly aggregated and with the aid of geostatistical techniques the nematode intensities were mapped and the risk areas accurately identified. Consequently, geostatistics is here considered a useful tool for planning nematode control strategies, particularly in precision agriculture.

Key words: cotton, crop rotation, geostatistics, *Rotylenchulus reniformis*, spatial distribution.

Brazil is the sixth largest cotton producer in the world, with about 3% of global production. However, production of cotton in Brazil decreased by 50% from 1991 to 1998. Among the major causes for this decrease is the reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, that ranks first, or sometimes second to the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, in causing damage to the crop (Lordello, 1992). The control of cotton nematodes can be achieved by applying nematicides, but these chemicals are highly toxic to humans and their use, in larger areas, often has been demonstrated to be uneconomical. To solve this problem, according to the approach referred to as Precision Agriculture, it has been advised that nematicides should be applied only to certain crop areas where the target-nematode damage threshold has been exceeded (Wheeler *et al.,* 2000). For this, a practical and efficient means of studying the spatial distribution of the nematodes should be available.

Nematodes are known to have an aggregated distribution, which has often been described using the negative binomial distribution (Goodell & Ferris, 1980). However, mathematical limitations occur with the negative binomial since the variance is not independent of the mean (Taylor, 1984). Several investigators (Boag & Topham, 1984; Boag *et al.*, 1987; Ferris *et al.*, 1990; McSorley & Dickson, 1991) used Taylor's Power Law to measure nematode aggregation and design sampling methods, but drawbacks have been associated with the use of this Law (Routledge & Swartz, 1991; Marshall *et al.*, 1998). Conventional statistical methods usually are not useful to describe data that are correlated in terms of spatial distribution. Geostatistics provide appropriate methodology to analyse spatially correlated data (Clark, 1979). Geostatistical techniques permit the quantification of spatial dependencies between field samples, providing that nematode intensities can be mapped in the area (Marshall *et al.*, 1998).

These three-dimensional contour maps prepared by the so-called kriging method facilitate the identification of the crop areas where the population density of the target species is higher and can then be related to similar maps *e.g.* of crop yield (Boag *et al.*, 1992), thus facilitating nematode control with nematicides to be achieved at an economic level. Geostatistical techniques have been recently applied to nematode data (Caswell & Chellemi, 1986; Chen & Bird, 1992; Webster & Boag, 1992; Wallace & Hawkins, 1994; Robertson & Freckman, 1995).

This geostatistical study was undertaken to investigate the spatial distribution of the reniform nematode on cotton cultivated under a crop rotation scheme.

MATERIALS AND METHODS

Field location and selected crops. The experimental area, monocultivated with cotton (*Gossypium hirsutum* L.) for more than 20 years, was located near Jaboticabal, São Paulo State, Brazil (21°15' S, 48°18' W), in a dusky (Oxisol) Latosol soil. The crops selected for rotation were: sorghum (*Sorghum vulgare* Pers.); peanut (*Arachis hypogaea* L.); and velvetbean [*Mucuna pruriens* (L.) DC. var. *utilis* (Wallitch ex Wight) Bak. ex Burck.]. The selection was based on literature data from which these crops were rated as unsuitable hosts to *R. reniformis* and on the operational facilities that such a rotation scheme could provide to the grower.

Nematode sampling and extraction. Two 32 x 48 m-sampling grids (A and B) were established, each one composed of sixty-four 4 x 6 m-plots (8 columns and 8 rows) numbered from 1 to 64. The grids were located adjacent to each other. For all crops, a soil sample (300 cm^3) was taken at the center of each plot at planting time (Pi) and at harvest (Pf). A centrifugal flotation technique (Jenkins, 1964) was used for nematode extraction.

Economical threshold level. The economic threshold level adopted was 250 nematodes per $300cm^3$, assuming a soil bulk density greater than or equal to 1.2 g/cm^3 . This value was based on the threshold level of 100 nematodes per 100g soil previously suggested for the interaction cotton x *R. reniformis* (Starr & Page, 1990).

Geostatistical analyses. The analyses were carried out using GSLIB software (Deutsch & Journel, 1992). For the statistical procedures described here it is assumed that the sample spatial position and the nematode count for that location had been used in the analysis. Aggregated data, such as in the spatial distribution of nematodes, have been reported to have spatial dependence (Webster & Boag, 1992; Wallace & Hawkins, 1994). The spatial dependence between neighboring samples/counts can be measured with the semivariogram (Vieira *et al.*, 1983) estimated as:

$$\gamma^*(h) = \frac{1}{2\,N(h)}\sum_{i=1}^{N(h)}[Z(x_i) - Z(x_i + h)]^2$$

where $N(h)$ is the total number of pairs of nematode counts that are separated by a distance h. A graph of $\gamma^*(h)$ versus the corresponding values of h, called semivariogram, is a function of the distance h, and, therefore, depends on both its magnitude and direction. A mathematical model equation needs to be fitted to the semivariogram so that the expression of the spatial dependence can be used for estimation of values for unsampled locations. For properties that are spatially dependent, it is expected the increment $[Z(x_i)-Z(x_i+h)]$ will increase with distance, up to some distance beyond which it stabilizes at a *sill* value, which has a symbol C and is numerically almost equal to the variance of the data. This distance is called *range* (a) and represents the radius of a circle within which the observations are so similar they are correlated. The semivariance value at the intercept to the $\gamma^*(h)$ axis is called *nugget effect* (C_0) and it represents the variability at spaces smaller than the minimum sampling distance. Therefore, the higher the value of the nugget effect with respect to the sill, the least spatial dependence has the variable. A comparison of the semivariogram parameters for different situations can provide important information on the corresponding spatial distribution. For instance, the ratio between C_0/C provides an estimation of the amount of randomness that exists in the data at spaces smaller than the sampling distance.

Frequently, it may be of interest to further develop modeling of the spatial structure. One such instance is when estimation for the unsampled locations is of interest in order to construct a detailed and precise map of the variable being studied. In this case, it is necessary to interpolate between the sampled points. Thus, if an estimation, z^*, is to be made for any location, x_0, where there are no measured values, as a linear combination of the neighboring measured values, it can be written as:

$$z^*(x_0) = \sum_{i=1}^{N}\lambda_i\, z(x_i)$$

where N is the number of measured values, $z(x_i)$, involved in the estimation, and λ_i are the weights associated with each measured value. If the spatial correlation expressed through the semivariogram is

Table 1. Semivariogram parameters (including model, sill, nugget and range) and R^2 and ratio $C_0/(C_0+C)$ values in the grids A and B.

Crop		Parameters of semivariogram			R^2	Model	$C_0/(C_0+C)$
		C_0	C	a (m)			
				Grid A			
Sorghum	Pi	2500000	14000000	13	0.59	Spherical	0.15
	Pf	80000	294000	15	0.69	Spherical	0.21
Peanut	Pi	53000	0	0	–	Random	1.00
	Pf	10600	0	0	–	Random	1.00
Velvetbean	Pi	8500	0	0	–	Random	1.00
	Pf	9000	15000	15	0.73	Spherical	0.38
Cotton	Pi	4200	6300	12	0.72	Spherical	0.40
	Pf	130000	350000	17	0.66	Spherical	0.27
				Grid B			
Sorghum	Pi	40000	545000	18	0.78	Spherical	0.07
	Pf	22000	33000	10	0.52	Spherical	0.40
Peanut	Pi	12000	19000	21	0.85	Spherical	0.39
	Pf	3700	0	0	–	Random	1.00
Velvetbean	Pi[a]	–	–	–	–	–	–
	Pf	320	0	0	–	Random	1.00
Cotton	Pi	6000	0	0	–	Random	1.00
	Pf	300	2100	16	0.61	Spherical	0.13

[a] *Rotylenchulus reniformis* was not detected in the soil samples.

used to define the values of the weights, λ_i, then the estimation process is called *kriging*. This estimation is unbiased and has minimal variance (Journel & Huijbregts, 1978).

RESULTS

Except for some situations where the nematode was distributed randomly, all the distributions fitted a spherical model (Table 1), and had ratio C_0/C values ranging from 0.07 to 0.4. These ratios were much lower than the value of 80%, beyond which, according to Journel and Huijbregts (1978), there is little difference between geostatistics and conventional statistics.

Omnidirectional semivariograms were calculated resulting in a set of distances and number of pairs as: 4 m (56 pairs), 7 m (202 pairs), 10 m (84 pairs), 13 m (314 pairs), 16 m (148 pairs), 19 m (284 pairs), 22 m (86 pairs) and 25 m (314 pairs). The number of pairs was higher than 30, which is the value recommended as a minimum necessary for semivariogram calculation (Guerra, 1988). The spherical model best fitted all the experimental semivariograms, as judged by the R^2 values. The R^2 values were relatively low (Table 1), reflecting the high fluctuation of the semivariances around the general spherical behavior.

Figure 1a shows the semivariogram for the Pi data on sorghum in grid A, with the spherical model fitted with a range of 13 m. The kriging map for these Pi values (Fig. 2A) showed the various different areas where the economical threshold level had been reached. The high nematode population densities shown in this figure reflected the result of 20 years of local continuous cotton cropping. The field was almost fully infested with *R. reniformis*, the population level reaching 19,000 nematodes per 300 cm^3 soil in some locations. The threshold level was reached in only two small areas. The semivariogram for the Pf on sorghum in grid A is shown in Figure 1b, with the spherical model fitted with a range of 15 m. The corresponding map is shown in Figure 2B. It is apparent that there was a very significant decrease in the nematode population from 19,000 to about 2,400 specimens per 300 cm^3 soil. The location of the highly infested areas did not appreciably alter under sorghum. The semivariogram for the Pi in grid B is shown in Figure 1c. The spherical model fitted indicated that the spatial distribution of the nematode was not random, but rather showed a clear spatial dependence. The corresponding map for grid B (Fig. 3A) showed two areas where the economic threshold level had been reached. The semivariogram for the Pf data (Fig. 1d) fitted to the spherical model with a very weak spatial dependence. In the map (Fig. 3B) it is apparent that there was a marked decrease in the population

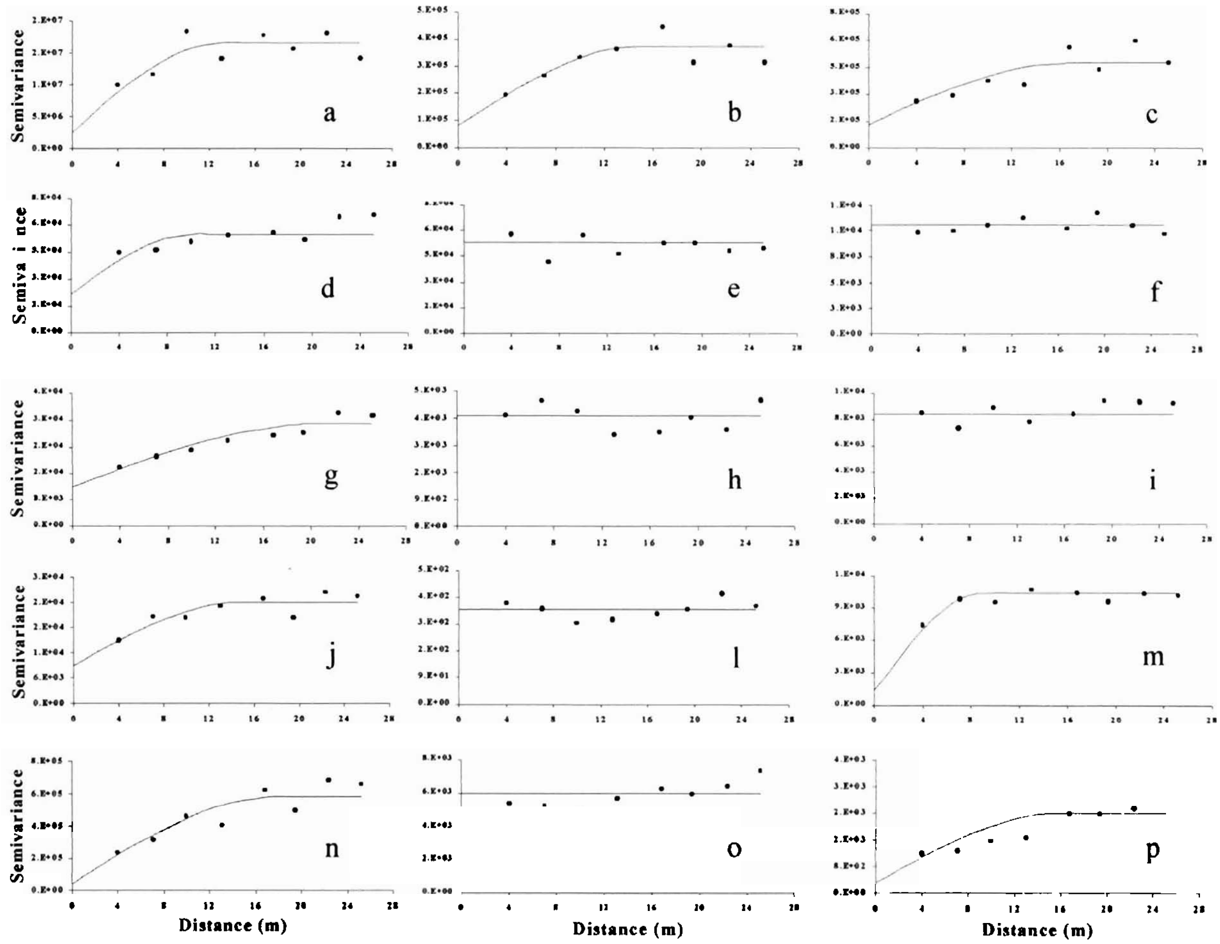

Fig. 1. Semivariograms for the *Rotylenchulus reniformis* initial (Pi) and final (Pf) population data on sorghum (Grid A: a-Pi, b-Pf; Grid B: c-Pi, d-Pf), peanut (Grid A: e-Pi, f-Pf; Grid B: g-Pi, h-Pf), velvetbean (Grid A: i-Pi, j-Pf; Grid B: l-Pf) and cotton (Grid A: m-Pi, n-Pf; Grid B: o-Pi, p-Pf).

density, the presence of the nematode being confined to five small areas.

The Pi and Pf data on sorghum for grids A and B revealed that there was a significant decrease in the nematode population. However, the population was still distributed over a large area of the field. These results confirmed that sorghum is an unsuitable host for *R. reniformis* (Thames & Heald, 1974; McSorley & Parrado, 1983; Rodríguez-Kábana *et al.*, 1998) but a single crop did not result in nematode eradication.

The spatial distribution of *R. reniformis* on peanut in grid A was completely at random, both for the Pi and Pf counts. Figures 1e and 1f showed the pure nugget effect semivariograms. As there was no spatial dependence, kriging maps were not constructed for this grid. However, the maximum and minimum values for Pi and Pf (Table 1) indicated a significant decrease in the nematode population. The semivariogram for Pi on peanut in grid B (Fig. 1g) fitted the spherical model, with a weak spatial dependence as indicated by the small difference between the nugget effect and the sill values. The corresponding map (Fig. 3C) showed two areas where the nematode population was concentrated with a small decrease from the Pf on sorghum to the Pi on peanut. The final population on peanut in grid B was completely random, as shown by the semivariogram (Fig. 1h). However, as for grid A, it can be seen that there was a decrease in the population level (Table 1). Peanut has also been rated as an unsuitable (but not immune) host for *R. reniformis* (Birchfield & Brister, 1962) with a few mature female specimens being found attached to the plant roots.

The semivariogram for the Pi data on velvetbean in grid A (Fig. 1i) showed that the distribution was completely random and thus the kriging map was not constructed. The nematode population was reasonably stable between the Pf on peanut to the Pi on velvetbean (Table 1). This indicates that peanut acts as a host only for a few

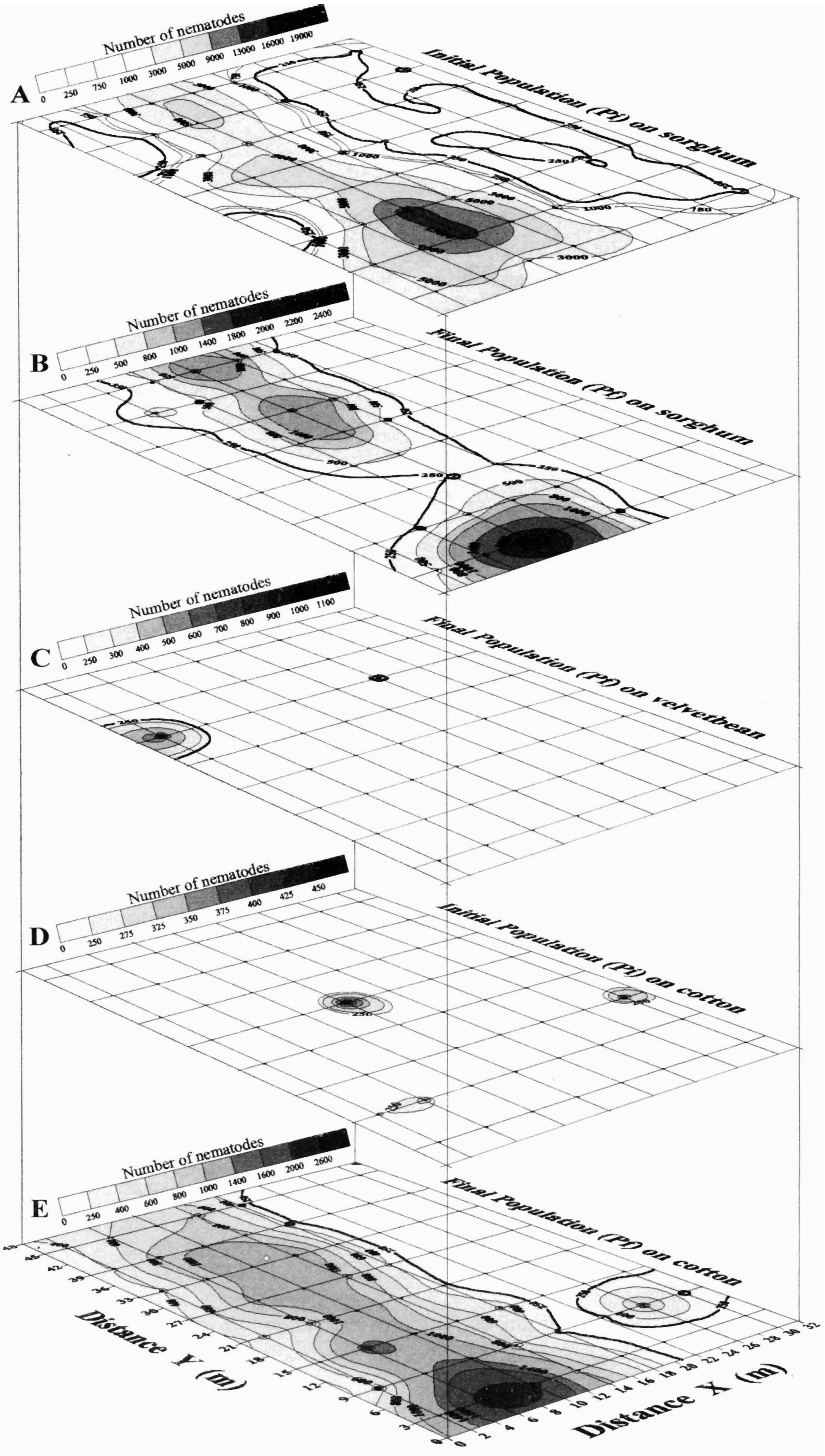

Fig. 2. Kriging maps of the spatial distribution of *Rotylenchulus reniformis* in grid A: on sorghum (A: Pi and B: Pf); velvetbean (C: Pf); and cotton (D: Pi and E: Pf).

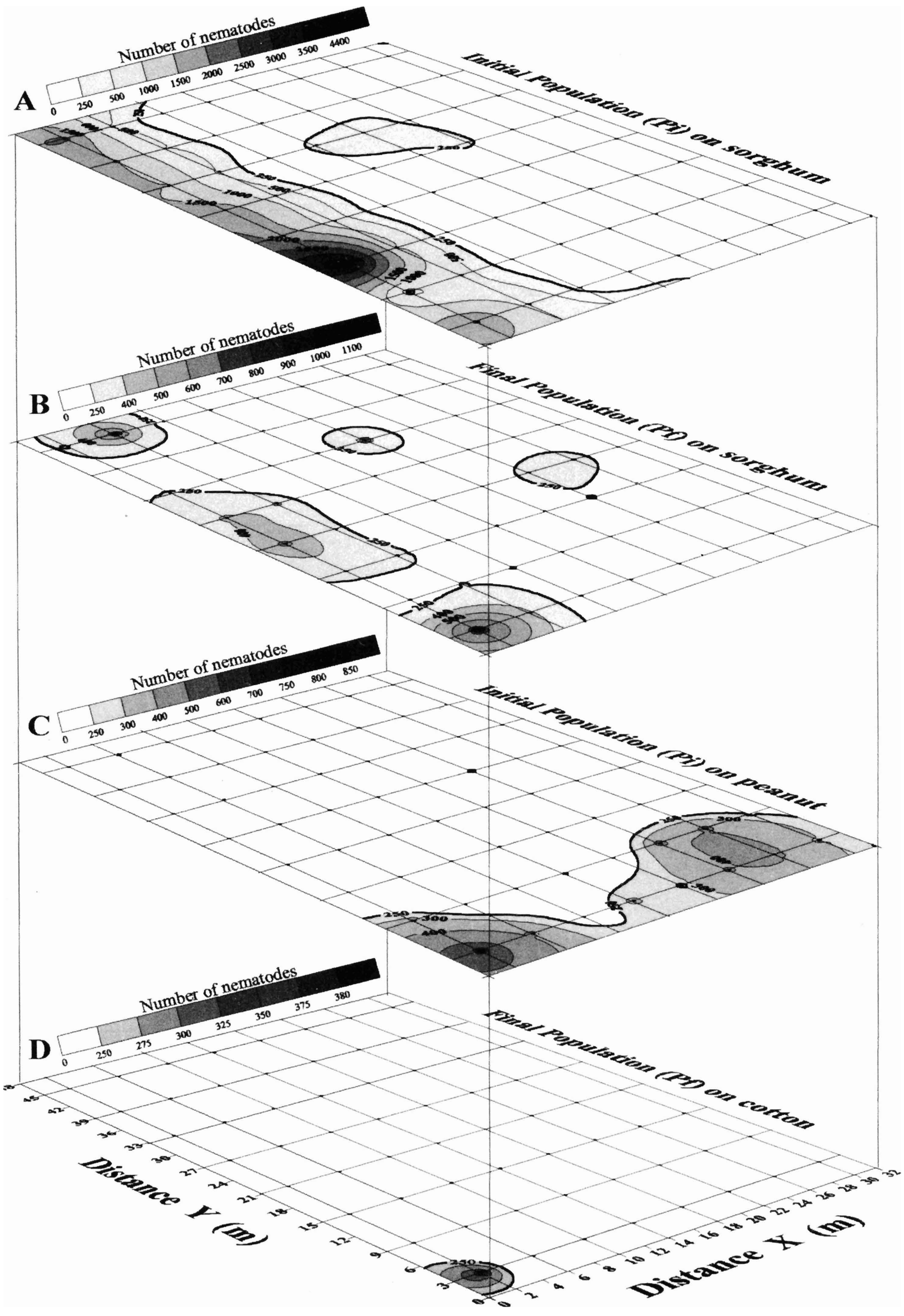

Fig. 3. Kriging maps of the spatial distribution of *Rotylenchulus reniformis* in grid B: on sorghum (A: Pi and B: Pf); peanut (C: Pi); and cotton (D: Pf).

nematode specimens. A spherical model was fitted to the semivariogram of the Pf data (Fig. 1j). In the corresponding map (Fig. 2C), the nematodes were concentrated in two areas. In grid B, nematodes were not detected at initial sampling (Pi). The Pf had a pure nugget effect semivariogram indicating that the spatial distribution was completely random (Fig. 1l). The maximum and minimum values recorded (Table 1) revealed that one growing season with velvetbean allowed the nematode population to be kept at a low level. The benefits of using velvetbean, a tropical leguminous forage crop, as a rotation crop against *R. reniformis* and other important plant-parasitic nematodes, e.g. *Meloidogyne incognita*, has previously been demonstrated (Quénéhervé *et al.*, 1998; Rodríguez-Kábana *et al.*, 1998).

The semivariogram for the Pi data on cotton in grid A (Fig. 1m) fitted best to a spherical model. The map (Fig. 2D) showed that the number of nematodes reached the threshold value in three well-defined small areas. In a Precision Agriculture context, control measures should be recommended for these localized areas, for example by applying nematicides. The semivariogram for the Pf (Fig. 1n) also fitted best a spherical model. The map (Fig. 2E) showed a marked and rapid increase in the nematode population under the cotton season, indicating that to reduce the parasite population the host plant is a key factor. In grid B, the Pi had a pure nugget effect semivariogram (Fig. 1o) indicating that the spatial distribution was completely random. A kriging map was not constructed for these data. The maximum and minimum values (Table 1) showed an increase in the nematode population in relation to the previous crop, confirming that cotton is a suitable host for *R. reniformis*. The semivariogram for Pf (Fig. 1p) expresses an apparent trend in the data. However, when the trend was removed using a least square fitted trend surface, the resulting semivariogram did not improve the kriging estimations. For this reason, the original semivariogram was used. The map (Fig. 3D) showed only one isolated infested area in the field, where the count for *R. reniformis* was about 380 nematodes per 300 cm^3 soil.

DISCUSSION

The spherical model best described the spatial distribution of the reniform nematode, with an average range of 15 m. Caswell & Chellemi (1986), studied the spatial distribution of the same nematode species on pineapple in Hawaii and concluded that the distribution was strongly aggregated. Also, these authors suggested that the semivariogram spherical model best described the spatial distribution of the nematode, with a dependence range of 10 m. In other recent studies dealing with the geostatistical analysis of the spatial distribution of plant-parasitic nematodes (Chen & Bird, 1992; Webster & Boag, 1992; Wallace & Hawkins, 1994) the same trends, i.e. strong nematode aggregation and semivariograms fitting well to the spherical model, have been reported.

Figures 2A, 2E and 3A showed an increase in the nematode population near the y axis following the direction of the contour levels. This was probably due to soil tillage that spread the nematode over the field.

Grid A best represented the spatial distribution of *R. reniformis*, showing higher population densities than grid B. In grid A, the low values determined for the nematode Pi on cotton (Fig. 2D) reflected the benefits of the crop rotation system used locally. Sorghum, peanut, and particularly velvetbean proved to be unsuitable hosts for reniform nematode and thus provide good cultural control. However, this kriging map also showed that crop rotation did not result in nematode eradication. On the contrary, the parasite still occurred in three small aggregated areas, thus probably providing foci for rapid spread throughout the field during subsequent cotton crops. Consequently, it should be recommended to the grower that nematicide be applied to the three areas where economic threshold levels had been reached, which represented only about 3% of the total cultivated area. In terms of a cost/benefit ratio, this simple procedure should provide a positive benefit. This particular case can be used to illustrate the relevance of the geostatistical techniques in studies dealing with the spatial distribution of *R. reniformis*, and many other plant-parasitic nematodes, providing the adequate means are available for the nematode-infested field to be mapped and the risk areas accurately assessed and identified. As emphasised by Wheeler *et al.* (2000), the collection and assay of a relatively high number of soil samples in nematode-infested areas for identification of the sites to be treated with nematicides may be an expensive proposition, with costs in the USA reaching US$25/sample. However, these authors verified that in *Meloidogyne incognita*-infested fields in Texas continuously cropped with cotton the results of an intensive sampling for assessment of the nematode distribution could be applicable for more than just one growing season (for the following two years at some sites). Thus, the expense could be better justified and these authors concluded that sampling frequency required to be decided on a field-by-field basis. Consequently, it can be assumed that any generalisation in regard to the feasibility or not of this methodology is speculative. For example, in Brazil where the costs of assays usually range from US$8 to 10/sample (less than in the USA), and cotton has been a high-value crop for many years, the regular application of this methodology can not be recommended. A further factor, the cost of nematicides needs to assessed. These chemicals are quoted in US dollars and Brazil is subject to fluctuations in its currency, therefore the cost of nematicide may become prohibitive to the grower necessitating nematode management strategies to be reevaluated.

Global Positioning System (GPS) and GIS (Geographic Information System) are currently used in Precision Agriculture, and together with geostatistical techniques may represent a useful means for improving certain studies of plant-parasitic nematodes, as demonstrated here in relation to the development of control strategies under particular field conditions.

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